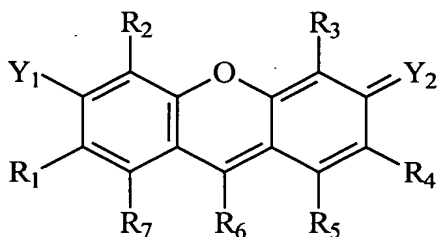


WE CLAIM:

1. An aromatic-substituted xanthene dye compound having the formula:



wherein:

Y₁ and Y₂ taken separately are selected from the group consisting of hydroxyl, oxygen, imminium, linking group and amine, or Y₁ taken together with R₂ is cyclic amine, or Y₂ taken together with R₃ is cyclic imine;

R₂, R₃, R₅, and R₇ taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, sulfone, amino, imminium, amido, nitrile, lower alkoxy, phenyl, and linking group;

R₁ taken separately is selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, and electron-rich heterocycle, or when taken together with R₇ is selected from the group consisting of electron-rich heterocycle and indene;

R₄ taken separately is selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, sulfone, amino, imminium, amido, nitrile, lower alkoxy, phenyl, linking group, amine, phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, indene, and electron-rich heterocycle, or when taken together with R₅ is selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, indene, and electron-rich heterocycle;

and

R₆ is selected from the group consisting of acetylene, lower alkyl, lower alkene, cyano, phenyl, substituted phenyl, heterocyclic aromatic, and combinations thereof, the substituted phenyl having the structure:

08/942,067

Benson

16-1-97

Tucox

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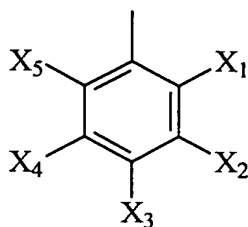
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wherein:

X_1 - X_5 taken separately are hydrogen, chlorine, fluorine, lower alkyl, carboxylic acid, sulfonic acid, $-CH_2OH$, or linking group.

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2. The dye compound of **claim 1** wherein R_1 taken together with R_7 is benzofuran having 2 and 3 positions fused to the xanthene ring.

3. The dye compound of **claim 2** wherein R_4 taken together with R_5 is benzofuran having 2 and 3 positions fused to the xanthene ring.

4. The dye compound of **claim 3** wherein R_2 and R_3 are hydrogen.

5. The dye compound of **claim 2** wherein R_4 is chloro, and R_2 and R_3 are hydrogen.

6. The dye compound of **claim 1** wherein R_1 and R_4 are selected from the group consisting of phenyl and substituted phenyl.

7. The dye compound of **claim 6** wherein R_2 and R_3 are hydrogen, and R_6 is a substituted phenyl wherein X_2 and X_5 are chloro, and X_1 is carboxylic acid.

8. The dye compound of **claim 1** wherein R_1 is selected from the group consisting of naphthyl and substituted naphthyl.

9. The dye compound of **claim 8** wherein R_4 is selected from the group consisting of naphthyl and substituted naphthyl.

10. The dye compound of **claim 9** wherein R_6 is a substituted phenyl wherein X_2 and X_5 are chloro, and X_1 is carboxylic acid.

11. The dye compound of **claim 10** wherein R_2 and R_3 are H.

12. The dye compound of **claim 10** wherein R_2 and R_3 are ^{Fluoro}~~fluoro~~.

13. The dye compound of **claim 1** wherein R_4 taken together with R_5 is phenyl or polycyclic aromatic.

14. The dye compound of **claim 13** wherein R_6 is a substituted phenyl wherein X_2 and X_3 are chloro, and X_1 is carboxylic acid.

15. The dye compound of **claim 14** wherein R_1 taken together with R_7 is benzofuran having 2 and 3 positions fused to the xanthene ring.

16. The dye compound of **claim 15** wherein R_2 is H and R_3 is fluorine.

17. The dye compound of **claim 14** wherein R_1 is ^{naphthyl}~~naphthyl~~.

18. The dye compound of **claim 17** wherein R_7 is H and R_2 and R_3 are fluorine.

19. The dye compound of **claim 1** wherein R_1 is phenyl, Y_1 is OH, Y_2 is O, R_4 is Cl, and R_6 is a substituted phenyl wherein X_2 and X_3 are chloro, and X_1 is carboxylic acid.

20. An energy transfer dye comprising:

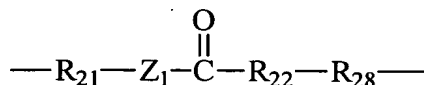
a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

an acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response; and

a linker for linking the donor dye and the acceptor dye, the linker serving to facilitate the efficient transfer of energy between the donor dye and the acceptor dye;

wherein at least one of the donor dye and acceptor dye is an aromatic-substituted xanthene dye of **claim 1**.

21. The energy transfer dye of claim 20 wherein the linker has the structure



wherein

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

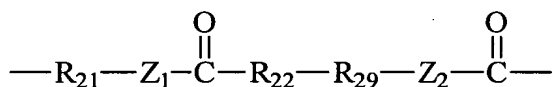
R_{21} is a lower alkyl attached to the donor dye;

R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond and a fused ring structure which is attached to the carbonyl carbon; and

R_{28} includes a functional group which attaches the linker to the acceptor dye.

22. The energy transfer dye of **claim 21** wherein R_{22} is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

23. The energy transfer dye of **claim 21** wherein the linker has the structure

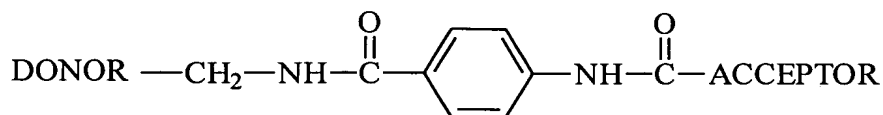


wherein

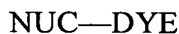
Z_2 is selected from the group consisting of NH, sulfur and oxygen; and

R_{29} is a lower alkyl.

24. The energy transfer dye of **claim 20** wherein the linker has the structure



25. A labeled nucleoside/side having the formula:



wherein

5 NUC is a nucleoside/tide or nucleoside/tide analog;

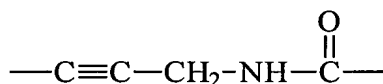
DYE is an aromatic-substituted xanthene dye compound of **claim 1**, NUC and DYE being connected by a linkage;

wherein the linkage is attached to DYE at one of positions $R_1 - R_6$; and

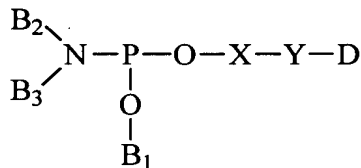
10 wherein if NUC comprises a purine base, the linkage is attached to the 8-position of the purine, if NUC comprises a 7-deazapurine base, the linkage is attached to the 7-position of the 7-deazapurine, and if NUC comprises a pyrimidine base, the linkage is attached to the 5-position of the pyrimidine.

26. The labeled nucleoside/tide of **claim 25** wherein NUC comprises a base selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

27. The labeled nucleoside/tide of **claim 25** wherein the linkage is



28. A phosphoramidite compound having the formula:



wherein:

X is a spacer arm;

Y is a linkage;

25 B_1 is a phosphite ester protecting group;

B₂ and B₃ taken separately are selected from the group consisting of lower alkyl, lower alkene, aryl, and cycloalkyl containing up to 10 carbon atoms; and

D is a dye compound of **Claim 1**;

wherein Y and D are linked through a linkage attached to D at one of positions R₁-

5 R₆.

29. The compound of **claim 28** wherein B₂ and B₃ taken together form an alkene chain containing up to 5 carbon atoms in the principle chain and a total of up to 10 carbon atoms with both terminal valence bonds of said chains being attached to the nitrogen atom; or B₂ and B₃ taken together with the nitrogen atom form a saturated nitrogen heterocycle which contains one or more heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur.

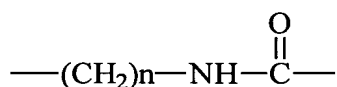
30. The compound of **claim 29** wherein:

B₁ is selected from the group consisting of methyl, β-cyanoethyl, or 4-nitrophenylethyl;

B₂ and B₃ taken separately are selected from the group consisting of isopropyl, t-butyl, isobutyl, and sec-butyl; and

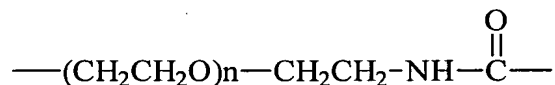
B₂ and B₃ taken together is morpholino.

31. The compound of **claim 28** wherein X and Y taken together is



wherein n ranges from 2 to 10.

32. The compound of **claim 28** wherein X and Y taken together is

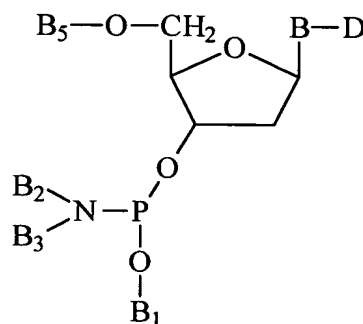


wherein n ranges from 2 to 10.

33. A phosphoramidite compound having the formula:

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T460X



wherein:

B₁ is a phosphite ester protecting group;

B₂, and B₃ taken separately are selected from the group consisting of lower alkyl,

5 lower alkene, aryl, and cycloalkyl containing up to 10 carbon atoms;

B₅ is an acid-cleavable hydroxyl protecting group;

B is a nucleotide base; and

D is the dye compound of Claim 1;

wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N⁹-
10 position of the purine or 7-deazapurine, and when B is pyrimidine, the sugar moiety is
attached at the N¹-position of the pyrimidine;

wherein B and D are linked through a linkage attached to D at one of positions R₁-
R₉; and

wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B
15 is 7-deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is
pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

34. The compound of **claim 33** wherein B is selected from the group consisting of
uracil, cytosine, deazaadenine, and deazaguanosine.

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35. A method of polynucleotide sequencing comprising the steps of:

forming a mixture of a first, a second, a third, and a ^{fourth} ~~forth~~ class of polynucleotides
such that:

each polynucleotide in the first class includes a 3'-terminal dideoxyadenosine and is
25 labeled with a first dye;

each polynucleotide in the second class includes a 3'-terminal dideoxycytidine and
is labeled with a second dye;

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each polynucleotide in the third class includes a 3'-terminal dideoxyguanosine and is labeled with a third dye; and ~~fourth~~ ^{Fourth}

each polynucleotide in the ~~forth~~ ^{Fourth} class includes a 3'-terminal dideoxythymidine and is labeled with a forth dye;

wherein one of the first, second, third, or ~~forth~~ ^{Fourth} dyes is an aromatic-substituted xanthene dye of **Claim 1**;

the other of the dyes being spectrally resolvable from the asymmetric benzoxanthene dye and from each other;

electrophoretically separating the polynucleotides thereby forming bands of similarly sized polynucleotides;

illuminating the bands with an illumination beam capable of causing the dyes to fluoresce; and

identifying the classes of the polynucleotides in the bands by the fluorescence spectrum of the dyes.

36. A method of fragment analysis comprising:

forming labeled polynucleotide fragments, the fragments being labeled with an aromatic-substituted xanthene dye of **Claim 1**;

subjecting the labeled polynucleotide fragments to a size-dependent separation process; and

detecting the labeled polynucleotide fragment subsequent to the separation process.

37. The method of **claim 36** wherein the size-dependent separation process is electrophoresis and the labeled polynucleotide fragment is detected by fluorescence.

add
C1